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High mobility group box-1 (hmgb1) in systemic vasculitides

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CHAPTER 2

HMGB1 in vascular diseases: Its role in vascular inflammation and atherosclerosis

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Abstract

The nuclear protein high mobility group box 1 (HMGB1) has been suggested to be involved in the pathogenesis of several vascular diseases such as systemic vasculitis and atherosclerosis. In systemic vasculitides including ANCA-associated vasculitis and Kawasaki disease, serum HMGB1 levels are higher in patients with active disease compared to healthy controls. In atherosclerotic disease, HMGB1 displays increased expression in nuclei and cytoplasm of macrophages and smooth muscle cells in the atherosclerotic lesions, and is implicated in the progression of the atherosclerotic plaque. Experimental models of acute coronary syndromes and cerebrovascular accidents show that HMGB1 is not only involved in the amplification of the inflammatory response during acute ischemic injury, but also in the recovery and remodeling process after ischemia. Patients with acute coronary syndromes or stroke present significantly higher serum levels of HMGB1 than healthy controls and levels are associated with disease severity and mortality. Here we review clinical and experimental studies dealing with the role of HMGB1 in vascular diseases.

1. Introduction

High mobility group box 1 (HMGB1) is a nonhistone DNA-binding protein of 215 amino acid residues organized into three domains that include two tandem HMG box domains (A box and B box) arranged in an L-shape configuration, and a 30 amino acids long C-terminal tail [1,2]. HMGB1 is constitutively expressed in most cell types and it resides mainly in the nucleus under physiologic conditions where it acts as a structural component in complex with chromatin. HMGB1 facilitates the assembly of nuclear proteins and participates in DNA replication, recombination, transcription and repair [3,4].

HMGB1 can be actively secreted from immunologically competent cells when exposed to microbe-associated molecular patterns (MAMPs), pathogen-associated molecular patterns (PAMPs) or cytokines such as tumor necrosis factor (TNF) α , interleukin (IL)-1 and interferon (IFN) γ [5,6]. HMGB1 is also passively released from necrotic, damaged cells or from apoptotic cells. In contrast to HMGB1 released from necrotic cells, HMGB1 released from apoptotic cells does not induce significant inflammatory responses. This is due to the oxidation of cysteine at position 106 in the HMGB1 molecule by mitochondrial reactive oxygen species released inside apoptotic cells. This oxidation precludes a significant pro-inflammatory response by HMGB1 [7].

Biologically active HMGB1 can be expressed on the plasma membrane or may be secreted into the extracellular milieu where it acts as a cytokine and interacts with the receptor for advanced glycation end products (RAGE) and toll-like receptors (TLR)2, TLR4 and TLR9 [4,8–11]. HMGB1 may also bind to other proteins like CXCL12, syndecan, triggering receptors expressed on myeloid cells 1 (TREM1), and macrophage adhesion molecule 1 (MAC1) [12–15]. Furthermore, HMGB1 is a part of the nucleic-acid-sensing system and binds to immunogenic nucleotides in order to activate innate immune responses during microbial infection and tissue damage [16]. The interaction with TLR4 has been shown to be dominant in inducing the release of cytokines such as TNF α , IL-1, IL-6 and IL-8 by activated macrophages via activation of the inhibitor of kappa B kinase complex (IKK), including the kinases IKK α and IKK β , leading to phosphorylation and degradation of the I κ B, nuclear translocation of NF- κ B, and enhanced expression of proinflammatory cytokine genes whose transcription is dependent on NF- κ B [9,17]. Several other biological activities of extra-cellular HMGB1 have been described related to targeting different cells involved in inflammatory and immune responses. Upon binding to monocytes, HMGB1 induces transendothelial migration to inflammatory sites. With respect to dendritic cells (DC), HMGB1 induces maturation of immature DC and migration to lymph

nodes and also expression of MHC class II molecules and secretion of several proinflammatory cytokines including IL-1 α , IL-6, IL-8, IL-12, TNF α and RANTES [18–20]. Ligation of HMGB1 to neutrophils induces cell activation through nuclear translocation of NF κ B, resulting in the production of cytokines such as TNF α and IL-8, activation of NADPH oxidase, increased adhesion, and chemotaxis [21–24].

Several effects of HMGB1 on T-lymphocytes have been described indicating that HMGB1 may also play a role in adaptive immunity [25]. HMGB1 has been shown to induce proliferation of CD3⁺ naïve T cells which is inhibited by anti-CD3 monoclonal antibodies. Th1 polarization has been demonstrated upon stimulation of DC and T cells by the B box of HMGB1 leading to the production of IL-2, IL-12 and IFN γ whereas polarization of CD4⁺ T cells into a Th2 phenotype has also been described [19,25–27]. Moreover, in vitro stimulation of CD4⁺ T cells from patients with rheumatoid arthritis with increasing concentrations of recombinant HMGB1 induced the production of IL-17 indicating that HMGB1 may contribute to Th17 activation in these patients [28]. Regarding regulatory T cells (Tregs), HMGB1 has been shown to decrease the expression of CTLA4 and FoxP3, and to inhibit the secretion of IL-10 leading to decreased regulatory T-cell activity [29,30].

HMGB1 has an effect on B cells as well, since it has been demonstrated that HMGB1 in DNA containing immune complexes can stimulate cytokine production through interaction with TLR9 and RAGE, activating plasmacytoid DC and B cells. Binding of HMGB1 to CpG oligodeoxynucleotides enhanced activation and cytokine production via RAGE and TLR9 [10]. Moreover, immune complexes containing HMGB1, DNA and IgG2a are also able to activate B cells through TLR9 in a RAGE-independent mechanism [31]. More recently, it has been demonstrated that HMGB1 is also released by activated plasma cells into the extra-cellular environment contributing further to the enhancement of inflammatory responses [32].

HMGB1 acts as a pro-inflammatory mediator that it is released after sterile injury or microbial invasion and activates immune competent cells to amplify inflammatory responses through the release of proinflammatory cytokines. The role of HMGB1 has been investigated in several systemic disorders such as sepsis, cancer, trauma, ischemia–reperfusion injury (e.g. stroke and acute myocardial infarction), acute respiratory distress syndrome, and chronic inflammatory and autoimmune diseases [11,33]. Here, we review the literature on circulating HMGB1, its expression in tissues, and its relation to specific disease manifestations and prognosis in inflammatory diseases of the vessel wall, in particular systemic vasculitis and atherosclerosis.

1.1. Methods to detect HMGB1 in serum, plasma and in tissues

HMGB1 can be detected both in serum and in plasma samples using a sandwich enzyme-linked immunosorbent assay (ELISA) or a Western blot technique [34]. HMGB1 levels may be 5 times higher when analyzed by Western blot technique compared to ELISA but levels correlated well between both assays [35]. The difference in HMGB1 levels between both techniques may be due to binding of HMGB1 to several molecules such as phospholipids, thrombomodulin and proteoglycans resulting in lower levels measured by ELISA [34,36,37]. In addition, Urbonaviciute et al. [38] demonstrated that serum and plasma components may interfere with the detection of HMGB1 by ELISA as HMGB1 may bind to plasma/serum proteins, in particular IgG class immunoglobulins. Titers of IgG in sera were shown to correlate inversely with the detected amounts detected by ELISA of a defined dose of recombinant HMGB1 added to these sera. IgG1 is the isotype predominantly found to co-immunoprecipitate with HMGB1 [38]. Immunohistochemical staining has been used to detect HMGB1 in tissues in studies that evaluated HMGB1 in chronic autoimmune and inflammatory diseases, liver transplantation, and malignant tumors. Using this technique, both the intensity of HMGB1 staining as well as

its localization can be evaluated, that is intranuclear, cytoplasmic or extracellular [35,39–43].

2. HMGB1 and anti-HMGB1 antibodies in systemic vasculitis

Serum levels of HMGB1 have been studied in several systemic vasculitides including Kawasaki disease (KD), Henoch-Schönlein purpura (HSP) and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) [44–49], but not in patients with large and medium-sized vessel vasculitis or cryoglobulinemic vasculitis. Antibodies against HMGB1 have been detected in patients with SLE and sepsis [33,50]. Sato et al., utilizing a Western blot technique, could not find those antibodies in 22 patients with AAV and 8 patients with HSP [48]. We have tested anti-HMGB1 antibodies using an in house ELISA technique and detected antibodies in 3 out of 24 patients with active AAV (12.5%) and in one of 18 healthy controls (5.6%) (unpublished data).

2.1. HMGB1 in Kawasaki disease

Serum levels of HMGB1 have been tested in two studies that included 63 Japanese patients with KD. Patients in the early acute phase of KD presented the highest levels of HMGB1, decreasing significantly during the late acute phase and convalescent phase of the disease (Table 1). Even in the latter phase, serum levels of HMGB1 in KD were higher than in healthy controls and were comparable to levels in patients with sepsis. Gene expression of RAGE and CD74, the invariant chain of class II major histocompatibility complex, in peripheral blood mononuclear cells was also higher in the acute phase of KD [44].

The use of high-dose intravenous immunoglobulin (IVIG) in combination with aspirin has been shown to decrease the frequency of coronary artery aneurysms in children with KD, especially in those with a good response to therapy [51]. Eguchi et al. showed that levels of HMGB1

were a potential marker of poor response to IVIG therapy in KD, since its levels were significantly higher in poor-responders to IVIG when compared to those who had a good response (Table 1). Using a receiver operating characteristic (ROC) curve, HMGB1 levels as measured by ELISA showed the largest area under the curve (0.852), and the optimal cut-off point for predicting poor responsiveness to IVIG was 2.4 ng/mL, showing a sensitivity of 86% and a specificity of 86% [45]. HMGB1 levels were shown to correlate with leukocyte counts, a known prognostic factor for poor responsiveness to high-dose IVIG in KD [45,52]. As such, levels may be a surrogate marker for poor responsiveness to IVIG in KD.

2.2. HMGB1 in ANCA-associated vasculitis

Some studies have evaluated serum HMGB1 levels and HMGB1 expression in tissues in patients with AAV. HMGB1 levels were shown to be higher in patients with AAV compared to healthy controls, in particular in patients with active granulomatosis with polyangiitis (GPA) (Table 1) [48,49]. HMGB1 levels correlated with the Birmingham Vasculitis Activity Score (BVAS) ($r=0.49$; $p<0.005$) in GPA, while in microscopic polyangiitis (MPA) no significant differences were observed when levels of HMGB1 were compared between patients with active disease, patients in remission or controls (Table 1). Serum HMGB1 levels as measured by ELISA were significantly higher in patients with GPA when compared to MPA, and, using ROC curve analysis, a cut-off value of 4.4 ng/mL of HMGB1 was found to discriminate GPA patients from MPA patients. Sensitivity and specificity for active GPA were 84% and 83%, respectively [49]. However, no significant differences in median HMGB1 levels were found among patients with GPA, MPA and Churg-Strauss syndrome (CSS) in another study (Table 1) [47].

The relationship between specific organ involvement in patients with AAV and serum levels of HMGB1 has also been investigated. Patients with AAV and active biopsy-proven nephritis presented higher serum levels of

HMGB1 when compared to those with inactive kidney disease and healthy controls [47]. Otherwise, levels of HMGB1 were significantly higher in patients with GPA and predominantly granulomatous inflammation than in those with predominantly vasculitic manifestations (Table 1). A positive correlation was found between HMGB1 serum levels and volumes of pulmonary granulomatous tissue as measured by chest computed tomography volumetry ($r=0.761$; $p=0.0017$), suggesting that granulomatous inflammation is an important source of HMGB1 in GPA [46]. Increased extra-nuclear staining of HMGB1 has been found by immunohistochemistry in kidney biopsies from patients with AAV and active nephritis when compared to those with inactive disease [47]. Furthermore, endonasal biopsies from patients with GPA showed marked nuclear, cytoplasmic and extracellular staining for HMGB1 in granulomas [46]. Hence, the inflammatory process in the kidneys and in granulomatous tissue resulting in activated, damaged and even necrotic cells seems to be the source of the increased cytoplasmic and extracellular localization of HMGB1 in AAV.

Although HMGB1 serum levels and extra-cellular expression of HMGB1 are higher in patients with AAV and biopsy-proven active glomerulonephritis than in those without renal inflammation, the extent of granulomatous inflammation seems to have a major impact on HMGB1 production and/or release, even when the disease is confined to the ENT region and lungs [46].

3. HMGB1 in atherosclerotic lesions

Atherosclerosis is an inflammatory condition that affects the arterial wall and is characterized by progressive thickening due to the accumulation of lipids [53]. The early event in the pathogenesis of atherosclerosis is endothelial dysfunction as a result of endothelial injury possibly caused by elevated and modified LDL, free radicals from cigarette smoking, diabetes mellitus, hypertension and other risk factors for cardiovascular disease alone

or in combination. Endothelial injury leads to compensatory responses that alter hemostatic properties of endothelium and result in increased endothelial permeability and adhesiveness [53]. The increased endothelial permeability allows the internalization of lipids into the intima. LDL particles become trapped in the arterial wall and trigger the secretion of chemotatic factors and expression of adhesion receptors by endothelial cells favoring monocyte recruitment, adhesion and migration into the vessel wall. In the intima layer, monocytes differentiate into macrophages and start internalizing lipids. Then, macrophages are transformed into foam cells which in turn release growth factors, cytokines, matrixmetalloproteinases (MMP) and reactive oxygen species [54,55]. Smooth muscle cells migrate into the intima and synthesize collagen, elastin and proteoglycans. In advancing lesions, a central lipidic or necrotic core is formed consisting of dying macrophages and extra-cellular lipids with formation of a fibrous cap [54,56]. The ultimate complication of atherosclerosis is disruption of the atherosclerotic plaque leading to thrombosis and to the clinical manifestations of atherosclerotic disease [54,56].

Table 1. Clinical studies evaluating HMGB1 levels in patients with inflammatory vascular diseases.

Diseases	Methods for HMGB1 detection	Remarks	Reference
Kawasaki's disease	ELISA	HMGB1 levels were higher in the early acute phase ($29.8 \pm 29.3 \text{ ng/mL}$) when compared to the late acute phase ($16.3 \pm 20.3 \text{ ng/mL}$, $p < 0.01$) and the convalescent phase ($12.3 \pm 21.8 \text{ ng/mL}$, $p < 0.01$).	[44]
	ELISA	Mean HMGB1 levels were higher in patients who did not respond to high-dose IVIG than in those who had a good response (6.0 ng/mL vs. 1.5 ng/mL , $p < 0.01$).	[45]
ANCA-associated vasculitis	Western blot	Higher HMGB1 levels were found in patients with biopsy-proven active nephritis than in patients with inactive disease ($120 \pm 48 \text{ ng/mL}$ vs. $78 \pm 46 \text{ ng/mL}$, $p = 0.01$). No significant differences were found in median HMGB1 levels among patients with GPA, MPA and CSS (95.8 ng/mL vs. 127.7 ng/mL vs. 112.9 ng/mL ; $p = 0.53$).	[47]
	ELISA	HMGB1 levels were higher in active in comparison to inactive GPA (11.6 ± 8.8 vs. $4.8 \pm 3.3 \text{ ng/mL}$, $p < 0.001$). No difference between active and inactive patients with MPA (2.6 ± 2.5 vs. $2.6 \pm 2.7 \text{ ng/mL}$, $p = 1.00$) and HC ($3.0 \pm 2.8 \text{ ng/mL}$, $p = 0.9$).	[49]
	ELISA	HMGB1 levels were higher in patients with GPA and predominantly granulomatous disease than in those with predominantly vasculitic inflammation ($6.4 \pm 4.5 \text{ ng/mL}$ vs. $3.8 \pm 2.8 \text{ ng/mL}$, $p = 0.01$).	[46]
Coronary artery disease	ELISA	HMGB1 levels were higher in patients with CAD in comparison to those without CAD either in non-diabetic (4.3 ± 10.4 vs. $1.74 \pm 2.0 \text{ ng/mL}$, $P < 0.001$) or in diabetic patients (7.6 ± 15.1 vs. $3.3 \pm 8.2 \text{ ng/mL}$, $p < 0.001$).	[80]
Acute coronary syndromes	Western blot	HMGB1 levels were higher in patients with ACS than in controls (159.0 ± 54.3 vs. $1.9 \pm 2.0 \text{ ng/mL}$, $p < 0.001$).	[79]
	ELISA	Patients with acute MI present higher HMGB1 levels in comparison to controls ($14.8 \pm 6.8 \text{ ng/mL}$ vs. $2.3 \pm 1.0 \text{ ng/mL}$; $p < 0.0001$).	[83]
	ELISA	Plasma HMGB1 levels were higher in patients with ACS who died when compared to surviving patients [$4.8 \text{ } \mu\text{g/L}$ (range $3.1\text{-}7.5$) vs. $2.9 \text{ } \mu\text{g/L}$ (range $2.6\text{-}3.2 \text{ } \mu\text{g/L}$), $p < 0.05$].	[82]
Stroke	Western blot	Higher HMGB1 levels in patients with stroke than in controls ($218 \pm 18.8 \text{ ng/mL}$ vs. $16.8 \pm 10.9 \text{ ng/mL}$, $p < 0.001$).	[79]
Intracerebral hemorrhage	Western blot	Higher HMGB1 levels in patients with a poor outcome than in those with a favorable outcome (221.4 ± 49.5 vs. $114.6 \pm 32.6 \text{ ng/mL}$, $p < 0.001$).	[104]

ACS – Acute coronary syndromes; CAD – Coronary artery disease; CSS – Churg-Strauss syndrome; ELISA – Enzyme-linked immune assay; GPA – Granulomatosis with polyangiitis; HMGB1 – High mobility group box 1; MI – Myocardial infarction; MPA – Microscopic polyangiitis.

In normal human aorta, HMGB1 is constitutively expressed in endothelial cells, smooth muscle cells and in CD68 positive macrophages

localized close to the intima as well as in microvessels within the adventitia [57]. In contrast to normal human arteries, in human atherosclerotic lesions from the aorta, carotid and coronary arteries the expression of HMGB1 is markedly increased in the nuclei and in the cytoplasm of macrophages and smooth muscle cells localized near the intima [57,58]. Intense HMGB1 expression has also been observed in areas adjacent to the necrotic core of atherosclerotic lesions [57].

HMGB1 may be released from several cell types in the atherosclerotic plaque including smooth muscle cells, endothelial cells, foam cells, macrophages and activated platelets [57,59,60]. Once released, HMGB1 induces several inflammatory effects on endothelial cells, smooth muscle cells and macrophages. Recombinant HMGB1 has been shown to activate vascular endothelial cells leading to expression and secretion of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, granulocyte colony stimulating factor (G-CSF), RAGE, TNF α , monocyte chemotactic protein 1 (MCP-1), IL-8, plasminogen activator inhibitor 1, and tissue plasminogen activator [61,62]. Regarding smooth muscle cells from atherosclerotic plaques, HMGB1 promotes their proliferation, migration to the intimal layer, their release of more HMGB1 as well as C-reactive protein, and their expression of MMP2, MMP3 and MMP9 [58,59].

The importance of HMGB1 in the development of atherosclerosis has been demonstrated in apolipoprotein E deficient mice fed with a high-fat diet. The administration of neutralizing monoclonal antibodies against HMGB1 attenuated atherosclerosis by 55%. Furthermore, anti-HMGB1 neutralizing antibodies led to a decrease in macrophage, DC, and CD4⁺ T-cell accumulation in atherosclerotic lesions, and to a reduced expression of VCAM-1 and MCP-1 [63].

Statins have been shown to attenuate the effects of HMGB1 on endothelial cells in two experimental studies. Yang et al. observed that atorvastatin is able to inhibit endothelial activation in vitro upon HMGB1

stimulation in a dose-dependent manner. In this in vitro study, incubation of endothelial cells with 10 μ M atorvastatin reduced the expression of ICAM-1 and E-selectin, and inhibited HMGB1-stimulated leukocyte adhesion to endothelial cells. Moreover, atorvastatin also suppressed HMGB1-induced TLR-4 expression and NF κ B nuclear translocation in endothelial cells [64]. Haraba et al. observed that HMGB1 serum levels were increased in golden Syrian hamsters with induced hyperlipidemia, and HMGB1 release and RAGE expression were increased in cultures of U937-cells exposed to hyperlipemic sera. In this study, fluvastatin reduced serum HMGB1 levels by 38.2% and led to a 1.46-fold reduction of HMGB1 mRNA expression in lung tissue [65].

In contrast, a beneficial effect of HMGB1 has been demonstrated after ischemic limb injury in diabetic and non-diabetic mice. HMGB1 expression was lower in ischemic limbs of diabetic mice and this lower expression was associated with a diminished perfusion recovery after injury. Administration of HMGB1 significantly improved blood flow and capillary density in ischemic muscles of diabetic mice and this beneficial effect was associated with an increased expression of vascular endothelial growth factor (VEGF) [66].

3.1. HMGB1 in acute coronary syndromes

3.1.1. HMGB1 in experimental studies of ischemic heart disease

HMGB1 has been implicated as an inflammatory mediator in ischemic heart disease responsible for increasing damage to myocardial tissue. Indeed, ischemia–reperfusion injury of the heart induced increased myocardial expression of cytoplasmic HMGB1 and apoptosis in vivo. In vitro, anoxia–reoxygenation challenge induced an increase in intra-cellular levels and extra-cellular release of HMGB1 in isolated cardiomyocytes. Myocardial apoptosis was decreased following administration of HMGB1 box A, an antagonist of functional HMGB1 cytokine activity [67]. In an experimental model of ischemia–reperfusion injury of the heart in mice, HMGB1 has been demonstrated to be overexpressed in infiltrating leukocytes in the myocardium

and in left ventricle tissue lysates in the early phase whereas HMGB1 mRNA levels remained high in injured myocardium up to 7 days after ischemia/reperfusion injury, demonstrating de novo local production (Fig. 1). The administration of recombinant HMGB1 to mice worsened myocardial injury whereas treatment with HMGB1 box A, a specific HMGB1 antagonist, reduced infarct size and markers of tissue damage. The administration of recombinant HMGB1 or HMGB1 box A to RAGE-deficient mice had no effect, indicating an important role of HMGB1–RAGE interaction in ischemia–reperfusion injury of the heart [68]. Although considered to be a mediator of myocardial injury in ischemic heart disease, several studies have demonstrated beneficial effects of HMGB1 in experimental models of myocardial infarction (MI). Kitahara et al. observed smaller infarcted areas, improved cardiac function and higher survival rates after induced MI in transgenic mice with cardiac overexpression of HMGB1 when compared to control mice. The transgenic mice released more HMGB1 into the circulation after ligation of the left anterior descending coronary artery. These findings suggest a beneficial effect of released HMGB1 on the heart after MI [69].

The administration of HMGB1 to the myocardium some hours after ischemic injury has also resulted in better outcomes in experimental studies. Limana et al. administered purified HMGB1 into the peri-infarcted left ventricle 4 h after permanent coronary artery ligation in C57BL/6 mice. The procedure resulted in the formation of new myocytes within the infarcted area in association with proliferation and differentiation of endogenous cardiac c-kit⁺ progenitor cells. HMGB1-treated mice demonstrated a significantly better recovery of cardiac performance in the following weeks in comparison to non-treated mice [70]. The administration of HMGB1 to the myocardium three weeks after coronary artery ligation has also been shown to have beneficial effects in the study performed by Takahashi et al. In this study, HMGB1 was injected intra myocardially three weeks after left coronary artery ligation in female Sprague–Dawley rats resulting in a significant improvement in left

ventricular ejection fraction as observed 28 days later in comparison to non-treated rats. Moreover, accumulation of inflammatory cells, mainly DC, in the peri-infarcted area, cardiomyocyte hypertrophy and extra-cellular collagen deposition were all attenuated following HMGB1 administration when compared to control treated rats [71]. Limana et al. also found favorable outcomes when HMGB1 was administered to the peri-infarcted region three weeks after coronary artery ligation in female C57BL6 mice. Four weeks after treatment, there was an improvement in left ventricular function as well as a reduction of left ventricle volume, an increase in infarcted wall thickness and a reduction in collagen deposition in the myocardium compared to mice injected with denatured HMGB1. Furthermore, cardiac regeneration was seen with an increase in c-kit⁺ cell number, newly formed myocytes, and arteriole length density. HMGB1 also led to an enhancement in collagenase (MMP2 and MMP9) activity and a decrease in tissue inhibitor of metalloproteinase-3 (TIMP-3) levels indicating increased collagenolytic activity [72]. The effect of HMGB1 on proliferation and differentiation of cardiac c-kit⁺ stem cells has been demonstrated to be indirectly mediated through paracrine stimulation of cardiac fibroblasts to produce several inflammatory cytokines and growth factors [73]. In terms of angiogenesis in response to ischemia, HMGB1 stimulates homing of endothelial progenitor cells to ischemic tissues as well as endothelial cell migration, sprouting and neovascularization (Fig. 1) [74–76].

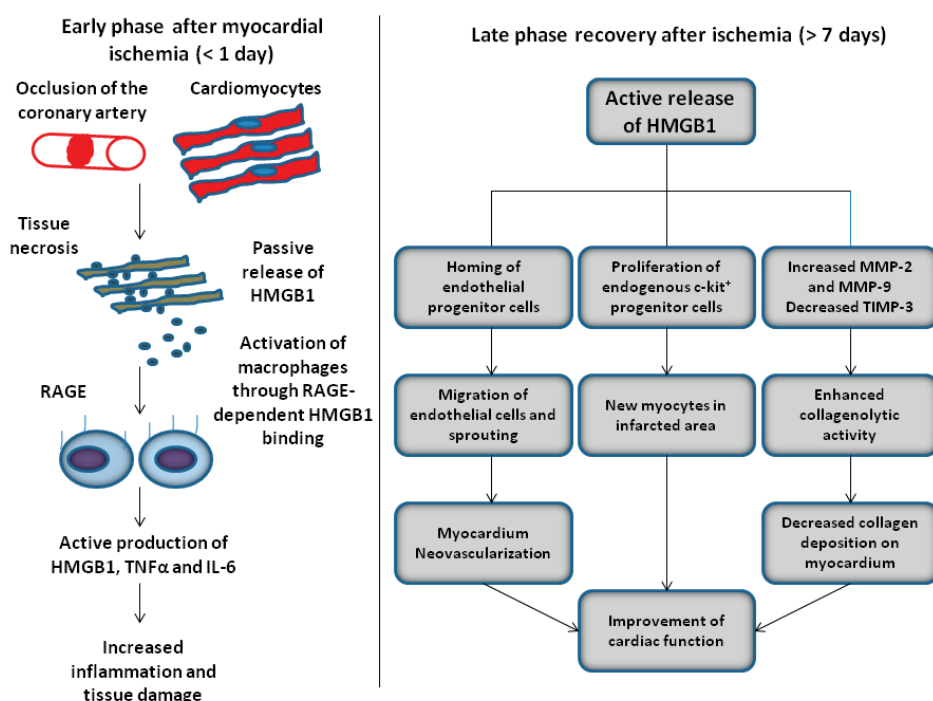


Figure 1. HMGB1 displays dual effects after myocardial ischemia. In the acute phase after ischemia HMGB1 is released by necrotic cells and activates macrophages via RAGE-dependent binding. Activated cells release HMGB1 and proinflammatory cytokines that amplify tissue damage caused by ischemia. HMGB1 mRNA levels remain high in injured myocardium up to 7 days after ischemia/reperfusion injury. In the late phase after ischemia, HMGB1 is essential for cardiac functional recovery contributing to tissue repair by stimulation of neovascularization, proliferation and differentiation of endogenous cardiac c-kit⁺ progenitor cells into myocytes and inhibition of excessive collagen deposition in the ischemic area.

More recently, Abarbanell et al. have shown a dose-dependent effect of HMGB1 on myocardial recovery after acute global ischemia/reperfusion injury in rat hearts using the Langendorff method. Either 200 ng or 1 μg of HMGB1 was administered 1 min after reperfusion and HMGB1 significantly improved myocardial functional recovery, decreased infarct size, and decreased levels of IL-1, IL-6, IL-10 and VEGF. Although HMGB1 dose

independently decreased myocardial inflammation and infarct size, it did not result in a dose dependent improvement of left ventricular function after ischemia/reperfusion [77].

The previous findings indicate disagreement regarding the role of HMGB1 in ischemic heart disease. Differences in study design and the dose of HMGB1 used in these studies may account for the opposing effects of HMGB1 in ischemic hearts. Nevertheless, the results of experimental studies indicate that HMGB1 is not only a proinflammatory mediator during the early phases of ischemia–reperfusion injury, but also acts as an important factor for recovery in MI in the later phases. In ischemia–reperfusion injury, especially after reperfusion, tissue injury is elicited by the release of oxygen-derived free radicals leading to inflammation and myocardial apoptosis, which are all enhanced by HMGB1 [78].

3.1.2. HMGB1 in clinical studies of acute coronary syndromes

Increased levels of serum HMGB1 in comparison to controls were reported for the first time in a small study that evaluated 9 patients with acute coronary syndromes (ACS). HMGB1 levels did not correlate either to creatine phosphokinase (CPK) or to troponin levels [79]. Next HMGB1 serum levels were evaluated in a large study that included type 2 diabetic and non-diabetic patients with or without coronary artery disease (CAD) regardless of symptoms of ACS. CAD was defined as a luminal diameter narrowed $\geq 50\%$ at a major epicardial coronary artery by angiography. HMGB1 levels were significantly higher in diabetic and non-diabetic patients with CAD compared to those without CAD (Table 1). A positive correlation between levels of HMGB1 and that of hsCRP, TNF α and IL-6 was found as well. Moreover, levels of endogenous secretory RAGE (esRAGE), a decoy receptor for advanced glycation end products and for HMGB1, were significantly lower in diabetic and non-diabetic patients with CAD. In multivariate regression

analysis, HMGB1 and esRAGE levels were independently associated with CAD either in diabetic or nondiabetic patients [80].

In another study that evaluated patients with ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI), HMGB1 serum levels were significantly correlated with infarct size as measured by cardiac magnetic resonance imaging performed 2–4 days after MI for STEMI and NSTEMI, respectively ($r^2=0.81$ and $r^2=0.74$; $p<0.001$). Furthermore, cardiac magnetic resonance imaging was repeated 6 months after MI to estimate residual ventricular function and showed an inverse correlation between HMGB1 levels during MI and the residual ejection fraction both in STEMI and NSTEMI, respectively ($r^2=-0.40$ and $r^2=-0.25$; $p<0.001$) [81].

HMGB1 plasma levels have also been shown to be associated with increased mortality in patients with STEMI due to occlusion of the left anterior descending coronary artery successfully treated with primary percutaneous coronary intervention. In this study, 144 patients were evaluated and 13 patients suffered cardiovascular death after a median 10 months of follow-up. The average baseline levels of HMGB1 were higher in patients who died compared to surviving patients (Table 1). A doubling in HMGB1 levels increased the risk of mortality (hazard ratio: 1.75; 95% confidence interval: 1.1 to 2.8) [82].

Cirillo et al. demonstrated that HMGB1 serum levels were higher in patients with acute MI than in controls or post-infarct patients (Table 1), HMGB1 levels at the time of MI were significantly correlated with cardiopulmonary parameters, such as oxygen consumption at peak exercise (VO_{2peak}) and the slope of increase in ventilation over carbon dioxide output (VE/VCO_{2slope}), as well as with Doppler echocardiographic parameters, peak creatine kinase-MB (CK-MB), and troponin I levels [83]. In this study, tissue factor pro-coagulant activity in vitro was progressively increased after

stimulation of human coronary artery endothelial cells with increasing doses of HMGB1 indicating that HMGB1 may have a role in inducing microvascular thrombosis [83]. In another study performed by the same group, post-infarct HMGB1 levels were lower in patients who underwent a 6-month exercise-based cardiac rehabilitation program. In trained patients, lower HMGB1 levels were significantly associated with improvement in VO₂ peak and heart rate recovery as well as with reduced left ventricular end-diastolic volume and wall motion score volume, indicating improvement in cardiopulmonary and autonomic function along with favorable cardiac remodeling [84].

Taken together, these studies have shown that levels of HMGB1 are higher in patients with subclinical CAD and in those with overt ACS than in controls. HMGB1 levels have also been shown to be a prognostic factor in ACS as HMGB1 levels correlate with larger infarct size, transmural, residual cardiac function, and mortality.

3.2. HMGB1 in ischemic stroke

Sudden arterial occlusion by thrombus formation or embolism leads to immediate loss of oxygen and glucose in cerebral tissue. This results in excitotoxicity, oxidative damage and neuronal death in the area affected by severe focal hypoperfusion while in the surroundings of the ischemic core, in the so-called penumbra area, neurovascular dysfunction is observed rather than neuronal death. Eventually, in the area affected by ischemic injury microvascular damage and blood–brain barrier dysfunction develop together with post-ischemic inflammation which may be detrimental and promote cell death in the early phases of stroke. HMGB1 can, however, also be beneficial and contribute to tissue remodeling during the recovery process [85,86].

3.2.1. HMGB1 in experimental studies of ischemic stroke

HMGB1 has been shown to be widely expressed throughout the normal rat brain, mainly in the nuclei of neurons and oligodendrocyte like cells

[87]. HMGB1 moves from the nucleus to the cytoplasm in neurons and astrocytes challenged with necrotic stimuli, and is then released passively into the extracellular space in the early phases after stroke [87,88]. Increased expression of HMGB1 is observed in the ischemic brain hemisphere and its levels increase as well in plasma and cerebrospinal fluid after an ischemic injury [89]. The immediate increase in extracellular HMGB1 is a result of passive release of HMGB1 due to excitotoxicity- and ischemia-induced neuronal death secondary to brain ischemia [88]. HMGB1 expression starts to decrease 3 h after middle cerebral artery occlusion (MCAO)/reperfusion and is further reduced one day after ischemic injury. Two days after MCAO/reperfusion, HMGB1 expression in brain tissue starts to increase again and it peaks around 4 days after MCAO/reperfusion [87]. This delayed expression of HMGB1 is observed in activated microglia, astrocytes and in microvascular structures, and results from active production of HMGB1 by activated cells (Fig. 2) [87–90].

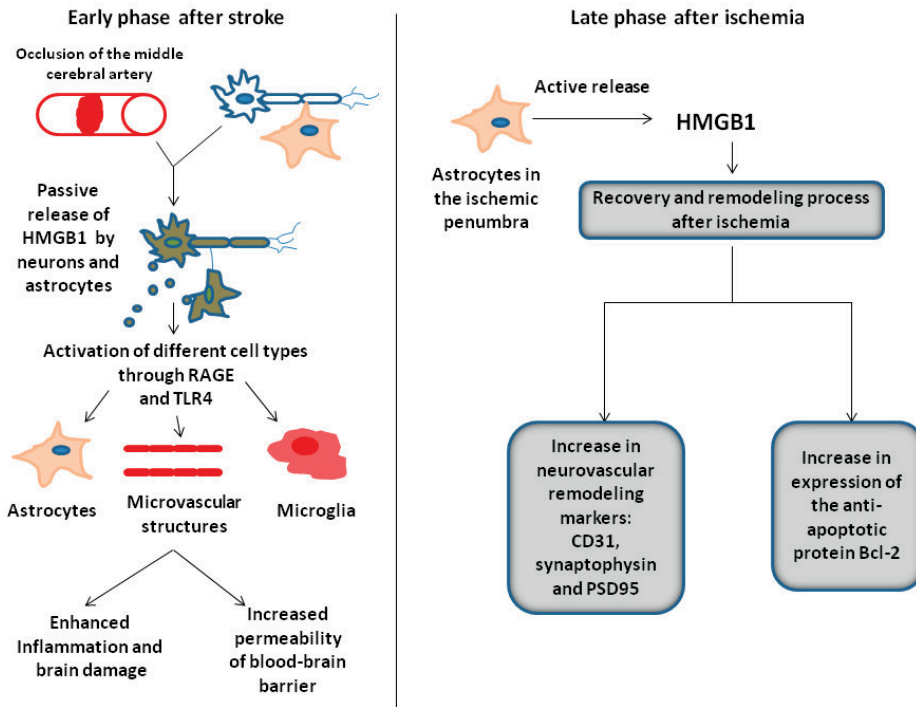


Figure 2. Passive release of HMGB1 stimulates inflammation in the acute phase and active release of HMGB1 stimulates recovery in the late phase after stroke. HMGB1 is passively released into the extracellular space by dying neurons and glial cells in the acute phase after ischemia. Extracellular HMGB1 activates several cell types through TLR4 and RAGE, especially the microglia, and enhances post ischemic inflammation and increased permeability of the blood–brain barrier. The acute effects of HMGB1 after brain ischemia lead to more brain edema and damage. In the late phase after brain ischemia, HMGB1 is released actively by activated astrocytes in the ischemic penumbra area. It contributes to the recovery and remodeling process of the brain through stimulation of vascular repair, neurite outgrowth and expression of Bcl-2 to protect neurons from apoptotic stimuli.

In the acute phase after stroke, the inflammatory process amplifies the initial ischemic injury and HMGB1 plays an important role in the induction of inflammation in ischemic brain tissue mainly through microglial activation, the hallmark of brain inflammation. RAGE and TLR4 have been shown to be the main receptors that exert HMGB1 effects in brain tissue affected by ischemia [90–92]. The importance of HMGB1 in the development of acute inflammation

after ischemic injury has been highlighted in studies that evaluated inhibition of HMGB1 with different agents. Blockade of HMGB1 signaling with short hairpin RNA in the post-ischemic brain suppressed infarct size, microglial activation and induction of proinflammatory mediators [89]. The use of anti-HMGB1 neutralizing antibodies in experimental models of MCAO/reperfusion led to a remarkable reduction in infarct size and an improvement in neurologic deficits in treated rats. Anti-HMGB1 antibodies also prevented the increase in permeability of the blood–brain barrier protecting the recipient from brain edema, inhibited activation of microglia and expression of TNF α and induced nitric oxide synthase (iNOS), while suppressing the activity of MMP9 [93,94]. Furthermore, the administration of several agents such as atorvastatin, minocycline, edaravone, cannabinal, niaspan and Tricin 7-glucoside was also shown to inhibit HMGB1 expression in brain ischemic tissue during the acute phase after stroke in experimental models of MCAO, so alleviating cerebral injury [95–100].

In the late phase after ischemia, recovery and remodeling take place in the brain. The interaction between neurons, glial cells, endothelial cells and extracellular matrix is important for neurovascular repair. In this recovery phase, reactive astrocytes not only contribute to glial scarring but also secrete several trophic factors that promote neurogenesis, synaptogenesis and angiogenesis after stroke [85]. It has been demonstrated that activated astrocytes concentrated in the ischemic penumbra area express HMGB1 2 days after stroke in parallel with an increase in neurovascular remodeling markers such as CD31, synaptophysin and PSD95. Metabolic inhibition of these astrocytes with fluorocitrate suppressed HMGB1 expression as well as neurovascular remodeling markers in astrocytes and resulted in worsening of behavioral recovery in mice after stroke [87,101]. These results indicate that HMGB1 produced by astrocytes is important for neurovascular repair in the brain after stroke. The HMGB1 receptor RAGE promotes neurotrophic effects in the nervous system upon stimulation by HMGB1 and S100 family proteins

that induce neurite outgrowth, activation of transcription factor NF κ B and increased expression of the anti-apoptotic protein Bcl-2 (Fig. 2). Nonetheless, hyperactivation of RAGE by high concentrations of its ligands promotes neuronal apoptosis [102].

In summary, experimental studies of stroke have shown that HMGB1 presents dual effects after ischemic injury in the brain. HMGB1 acts as a proinflammatory mediator in the acute phase that amplifies damage in ischemic tissue through the activation of microglia, enhancement of inflammation and increase of permeability of the blood–brain barrier. In contrast, in the late phase after ischemic injury HMGB1 contributes to the recovery and remodeling process stimulating neurovascular repair mainly by astrocytes in the affected brain.

3.2.2. HMGB1 in clinical studies of cerebrovascular accidents

In contrast to experimental studies of ischemic stroke, few studies have evaluated HMGB1 in patients with ischemic stroke. Higher HMGB1 levels were described in patients with cerebral vascular ischemia within 24 h after the onset of symptoms in comparison to control subjects (Table1) [79]. HMGB1 levels in patients with stroke remain significantly higher than in control subjects up to 14 days after the ischemic event while levels of the natural inhibitors of HMGB1, soluble RAGE (sRAGE) and esRAGE, remain indistinguishable from control subjects within 48 h following stroke. HMGB1 levels in patients with stroke are significantly correlated with IL-6 levels but not with the extent of brain tissue destruction as assessed by CT morphometry. Moreover, patients with stroke present an increased proportion of activated CD4⁺ T-cells in peripheral blood expressing CD25 or HLA-DR when compared to controls [103]. Due to the similarity between the kinetics of serum HMGB1 and the kinetics observed for the absolute number of CD4⁺ T-cells expressing HLA-DR, the authors raised the hypothesis that HMGB1 acts

as a link between brain tissue destruction by ischemic injury and the activation and Th1 priming of T-cells [103].

Zhou et al. observed higher levels of HMGB1 in patients with intracerebral hemorrhage (ICH) when compared to controls. HMGB1 was associated with the severity of stroke since patients with ICH and poor outcome had higher levels of HMGB1 than those with a favorable outcome (Table 1). There was a significant correlation between HMGB1 levels and the National Institutes of Health Stroke Scale (NIHSS) at day ten after stroke, and with the modified Ranking scale score at 3 months. HMGB1 was also correlated with IL-6 and TNF α levels in patients with ICH [104].

4. Conclusion

HMGB1 has been implicated in the pathogenesis of inflammatory vascular diseases including systemic vasculitis and atherosclerotic disease. High serum HMGB1 levels have been found in patients with ANCA-associated vasculitis and Kawasaki disease as a reflection of active disease. Furthermore, HMGB1 is expressed in atherosclerotic lesions by several cell types and contributes to the progression of the atherosclerotic plaque. HMGB1 levels are significantly increased in patients with subclinical CAD and in those who develop acute ischemic events in cardiac and cerebral vascular beds. Experimental studies show that HMGB1 has a dual effect, amplifying the inflammatory response as well as damage in the acute phase and participating in tissue remodeling during the late phase after ischemic injury. Targeting HMGB1 may be an attractive therapeutic modality for inflammatory vascular diseases.

Take-home messages

- HMGB1 levels are increased in the acute phase of Kawasaki disease and this has been regarded as a marker of poor response to intravenous immunoglobulin therapy.

- Increased levels of HMGB1 are detected in patients with ANCA associated vasculitis with active disease and this is correlated both with granulomatous manifestations and with biopsy-proven active renal involvement.
- HMGB1 is expressed in the majority of macrophages and in some intimal smooth muscle cells in atherosclerotic lesions, and is implicated in the progression of the atherosclerotic plaque.
- Deleterious and beneficial effects of HMGB1 have been described for HMGB1 in experimental models of atherosclerotic disease, since HMGB1 is involved in the amplification of the inflammatory response during acute ischemic injury but also in the recovery and remodeling process after ischemia.
- Serum HMGB1 levels are significantly higher in patients with acute coronary syndromes and stroke in comparison to control subjects.

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